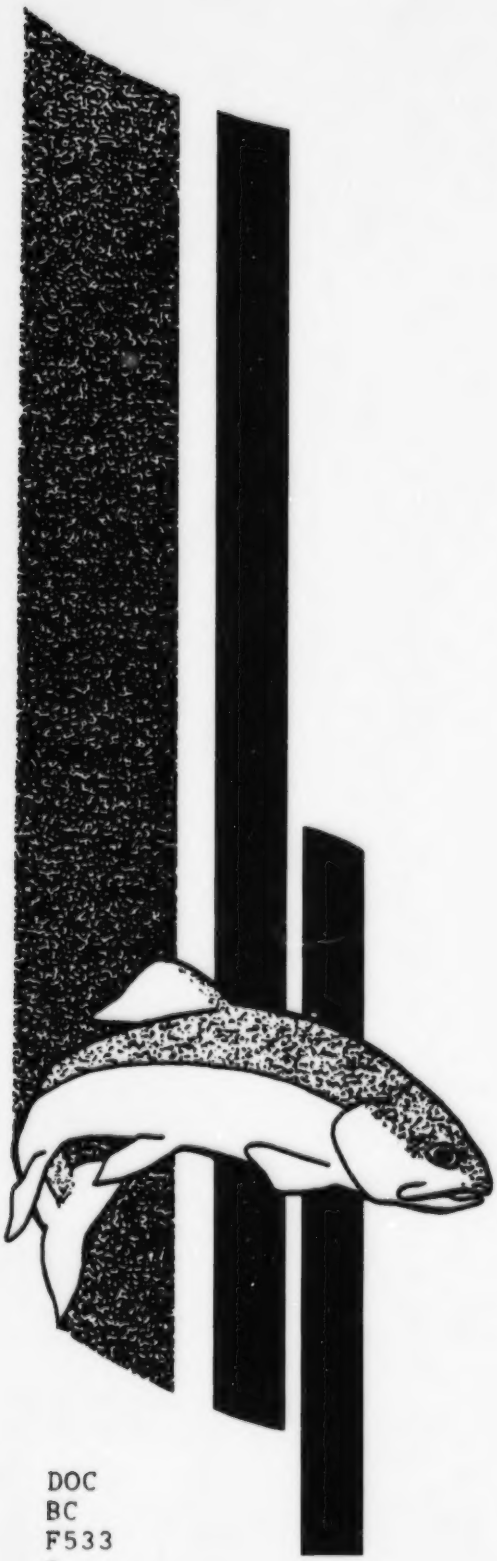

Phylogenetic Conservation Units for Rainbow Trout in British Columbia

by

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Within-species variation is a key concern for north temperate fish communities that are species-poor but rich in intra-specific variation as a result of repeated glaciations over the last two million years. In B.C., complex patterns of glacial coverage and freshwater refugia have resulted in major phylogenetic subdivisions in many of its native species. Phylogenetic divergence was documented in the rainbow trout, *Oncorhynchus mykiss*, using mitochondrial DNA (mtDNA) to address questions regarding its biogeographical history and conservation. A restriction fragment length polymorphism (RFLP) analysis was conducted of mtDNA, including populations throughout B.C. as well as from Alaska, Kamchatka, the northwestern U.S., and Alberta. Two distinct mtDNA lineages were revealed which overlap extensively in their geographical range. These data are combined with previous data on glacial history and molecular variation to generate a comprehensive hypothesis on the origins of rainbow trout populations in B.C. These data suggest that there were four, and possibly five, refugia where rainbow trout survived the last glaciation. Rainbow trout from a refuge in the Alaska-Kamchatka area and a hypothesized refuge east of the Rockies do not appear to have colonized B.C. To the south, however, there were separate refugia in the interior and coast from which rainbow trout colonized the southern interior and coastal regions of B.C. In the north there was a refuge near the Queen Charlotte Islands that was a main source for both northern coastal and interior populations.

Despite some ambiguity in the geographical boundaries of these phylogenetic groups, they represent the oldest genetic divergences within the species and, therefore, contribute significantly to the genetic resources of the species. Within phylogenetic groups, adaptive variation is also an important component of intraspecific variation. Conservation plans should recognize the phylogenetic subdivisions that are rare or threatened by development as well as the diversity of locally adapted populations within phylogenetic groups. Transfers between areas containing different phylogenetic groups should be avoided.

Acknowledgements

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INTRODUCTION

Conservation of biodiversity involves the protection of genetic diversity among populations within species as well as the protection of species and communities. This is particularly true for the freshwater fishes of B.C., where geology and history have combined to reduce the number of species and enhance the conditions for genetic divergence within species. Genetic diversity among populations can result from selective forces operating in different environments (ecotypic variation) or from ancestral differences that evolved in isolated source populations (phylogenetic variation). Due to the genetic diversity they represent, phylogenetic splits within a species should form the first level of intra-specific subdivision for conservation purposes (Utter *et al.*, 1993; Taylor and Haas, 1996).

In the Northern Hemisphere, the major events leading to phylogenetic divergence are associated with Pleistocene glaciations that repeatedly forced species into ice-free refugia (McPhail and Lindsey, 1986). B.C.'s mountainous terrain restricted dispersal both among the refugia as well as to the rest of the province following glacial retreats. Extended periods of isolation led to genetic distinction among these refugial groups due to a combination of mutation, genetic drift, and natural selection (Avise, 1994). The genetic divergences from the last glaciation and preceding ones are still very much present in the population genetic structure of many extant freshwater fish species (Bernatchez and Wilson, 1998).

Characterization of phylogenetic variation is increasingly done using molecular genetic markers (Bernatchez, 1995; Taylor and Haas, 1996). Similarities in these markers across contiguous geographic areas indicate a common ancestry among populations whereas differences among groups of populations indicate historical isolation. In addition, neutral genetic markers can trace historical events of population contraction and subsequent expansion due to recolonization. The resulting phylogenetic groups are thought to be important for conservation because they are likely to represent genetic differences throughout the genome of the organisms and, therefore, represent a significant portion of the genetic variation in the species. As genetic variation is what future selective pressure acts on, conservation plans are increasingly using phylogenetic groups to prioritize population units for protection (Allendorf and Leary 1988; Taylor and Haas 1996). In this report, we summarize the phylogenetic associations for BC rainbow trout populations and make recommendations on the conservation of this diversity. This summary is based on a recent study of DNA variation combined with older isozyme data, minisatellite data, and information on the geological history.

There are several possible source areas for rainbow trout populations that currently inhabit B.C. B.C. has been repeatedly glaciated for the last almost two million years, often with the same refugia present. During the last glaciation, the Cordilleran ice sheet covered the province of British Columbia, extending from Alaska south to the Olympic Peninsula, from approximately 60,000-10,000 years ago (McPhail and Lindsey, 1986). Several ice-free refugia have been hypothesized in and around British Columbia, which may have supported *O. mykiss* and other species. These include the Columbia River, the Chehalis River (just north of the Columbia River mouth), the Brooks Peninsula on northwestern Vancouver Island, the Queen

Charlotte Islands off the coast of central B.C., Beringia (Yukon River Valley and the exposed portion of the Bering Strait area), the Nahanni region in the Northwest Territories and northeastern B.C., and the Athabasca River in Alberta (Behnke, 1992; McPhail and Carveth, 1992).

Our objective was to test the hypothesis of Utter *et al.* (1980) that the rainbow trout are divided into two phylogenetic groups, a coastal group and an interior group, which presumably survived the last glaciation in a coastal and interior refuge. Since that time, Carl *et al.* (1994) have presented evidence that rainbow trout survived in a refuge east of the Rocky Mountains and Okazaki (1984) has suggested that Kamchatka trout as well as coastal trout in British Columbia survived in a northern refuge. In addition, we wanted to see if there was any evidence that rainbow trout occupied the hypothesized refuge on the north coast of BC near the Queen Charlotte Islands.

Mitochondrial DNA (mtDNA) variation is particularly suitable for addressing phylogenetic issues in freshwater fish. The high mutation rate of mtDNA relative to most nuclear loci and the effective population size one-quarter that of nuclear DNA make mtDNA a sensitive marker in intraspecific analyses. The lack of recombination in mtDNA makes interpretation of evolutionary relationships relatively straightforward (Wilson *et al.*, 1985; Avise, 1994). MtDNA variation has been widely assessed in north temperate freshwater fish species (Bernatchez, 1995, Taylor *et al.*, 1999). The term, Evolutionarily Significant Unit (ESU), was defined by Waples (1995) as a "substantially reproductively isolated group representing an important component of the evolutionary legacy of the species," is heavily dependent on molecular/biochemical data to assess the extent of reproductive isolation.

Though not universally accepted, the operational definition of ESUs in U.S. conservation plans for Pacific salmon and trout attests to the increasing reliance on molecular markers in management and conservation (Busby *et al.*, 1996; Myers *et al.*, 1998). For all the above reasons, mtDNA should be an excellent marker to address the biogeographical and conservation issues surrounding *O. mykiss*.

METHODS

Sample Collection

Samples were collected from every major watershed in British Columbia and from at least two sites in each of the Kamchatkan Peninsula in eastern Russia, Alaska, the Athabasca River in Alberta and the northwestern U.S. Samples were taken from several localities along each major river for the purpose of sampling a maximum amount of variation per watershed. Approximately five to ten sites were sampled per river, and no more than seven individuals were collected at each locality. In total, 292 samples from 59 sites were included in the composite RFLP analysis (Fig. 1) and an additional 219 samples from 56 sites were included in a diagnostic RFLP analysis (Fig. 2). The diagnostic analysis was done to increase sample size and to further resolve the geographical distributions of major clade groups.

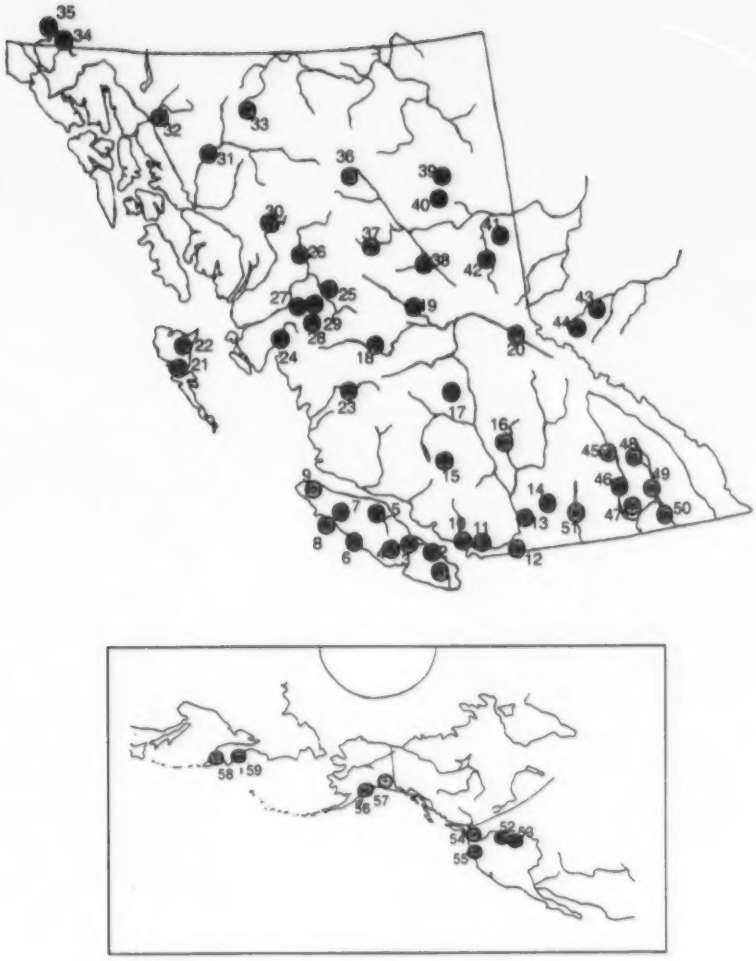

Figure 1. Composite haplotype sample sites in B.C. **Vancouver Island:** 1) Cowichan R., 2) Lucky Ck., 3) Elsie L., 4) Robertson Ck, 5) Quinsam R., 6) Bamfield, 7) Klootchlimmis Ck., 8.) Mahatta R., 9) Keogh R.; **Lower Mainland:** 10) Chehalis R., 11) Capilano R., 12) Vedder R.; **upper Fraser:** 13) Anderson Ck., 14) Pennask L., 15) Fish L., 16) Bonaparte R., 17) Tzenzaicut L., 18) Cheslatta L., 19) Bivouac Ck., 20) Dome Ck.; **Queen Charlotte Islands:** 21) Honna R., 22) BVM; **Central Coast:** 23) Dean R., 24) Kitimat R.; **Skeena:** 25) Morice R., 26) Babine R., 27) Canyon Ck., 28) Toboggan Ck., 29) Goathorn Ck.; **Nass:** 30) Meziadin R.; **North Coast:** 31) Tahltan R., 32) Taku R.; **Liard:** 33) Kutcho Ck.; **Yukon:** 34) McIntyre, 35) Kathleen R.; **Peace:** 36) Firesteel R., 37) Mesilinka R. 38) Nation R., 39) Chowade R., 40) Graham R., 41) Moose L., 42) Kiskatinaw R.; **Athabasca:** 43) Wampus Ck., 44) Halpenny Ck.; **Columbia:** 45) Tumtum Ck., 46) Hill Ck., 47) Halfway R., 48) Lardeau R., 49) Procter Ck., 50) Bayonne Ck.; **Okanagan:** 51) Goatskin Ck.; **Snake:** 51) Selway R., 52) Dworshak R.; **Puget Sound:** 54) Tahuya R.; **Oregon:** 55) Alsea R.; **Alaska:** 56) Natnek, 57) Karluk R.; **Kamchatka: 58)** Kavachina, 59) Snotolvayam

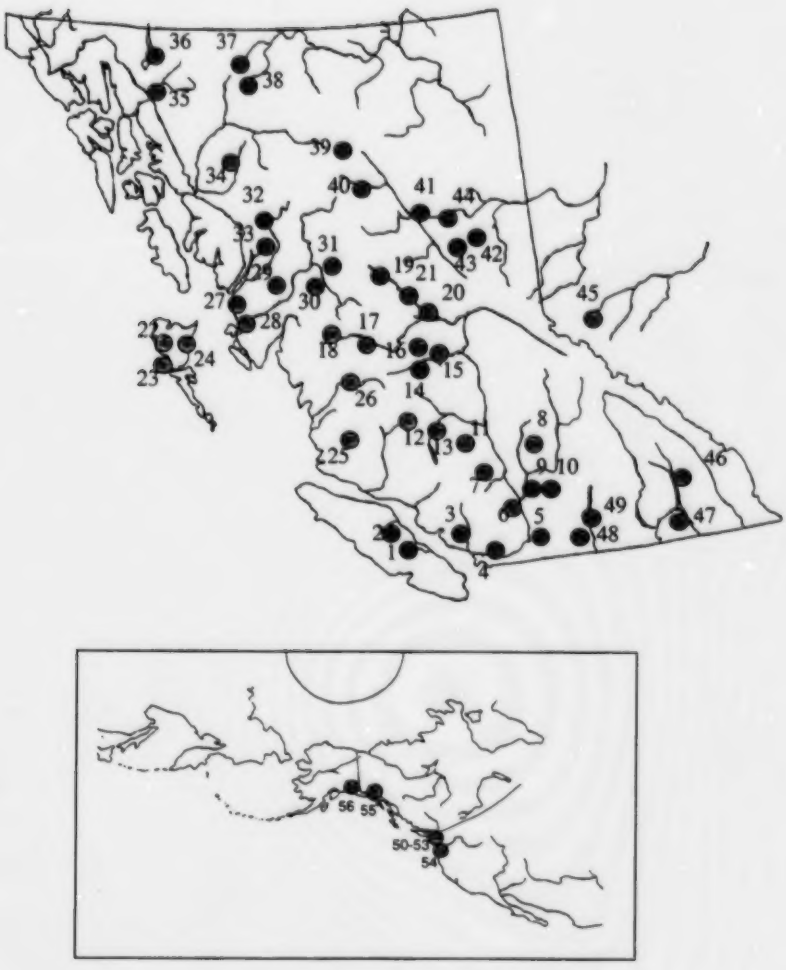

Figure 2. Diagnostic sample sites in B.C. **Vancouver Island:** 1) Junior L., 2) Efflingham R.; **Lower Mainland:** 3) Squamish R., 4) Abbotsford Hatch; **U. Fraser:** 5) American Ck., 6) Emory Ck., 7) Bridge R., 8) Deadman R., 9) Spius Ck., 10) Coldwater R., 11) Gaspard Ck., 12) Chilko/ Chilcotin R., 13) Puntzi Ck., 14) Euchiniko, 15) Baezaeko, 16) Coglistiko, 17) Brewer's Ck., 18) Tahtsa L., 19) Hautete Ck., 20) Baptiste Ck., 21) Forfar; **Queen Charlotte Islands:** 22) Brent Ck., 23) BVM, 24) L. Phantom Ck.; **Central Coast:** 25) Ashlulm Ck., 26) Abuntlet L., 27) Union, 28) Khtada; **Skeena**: 29) Kispiox R., 30) Sustut R., 31) Natlan Ck.; **Nass:** 32) Paw L., 33) Niska Ck.; **North Coast:** 34) Iskut R., 35) Taku R., 36) Disella L.; **Liard:** 37) Wolverine L., 38) Blue Sheep L.; **Peace:** 39) Firesteel R., 40) Ingenika R., 41) El Condor 42) Sekunka R., 43) Burnt R., 44) Elizabeth Ck.; **Athabasca:** 45) Cabin Ck.; **U. Columbia:** 46) Fry Ck., 47) Summit Ck.; **Okanagan:** 48) Goatskin Ck., 49) Fall Ck.; **Washington:** 50) Bogachiel R., 51) Nooksak R., 52) Deer Ck.; **L. Columbia:** 53) Tucannon R., 54) Lewis R.; **Alaska:** 55) Nushugak R., 56) Neguthluk R.

Fin clips were usually taken from the adipose fin, although muscle tissue was occasionally used. Electroshocking was the most common sampling technique, but some samples were collected by gillnet or angling. Sites where past introductions or transplants have been rumored or documented were avoided.

Restriction analysis

DNA was extracted from 20-30mg of tissue using a standard phenol/ chloroform extraction procedure (Taggart *et al.*, 1992). The regions of the mtDNA genome chosen for analysis were the D-loop, cytochrome b, and NADH dehydrogenase subunits 5 and 6 (ND5/6) as they have all been shown to be variable in salmonid fishes (Park and Moran, 1994). DNA was amplified using the Polymerase Chain Reaction, PCR. The primers used to amplify the D-loop and cytB regions were HN20 and C-Glu (Bernatchez and Danzmann, 1993), and the primers for the ND5/6 region were C- Glu and C-Leu-3 (Park *et al.*, 1993). The PCR conditions for both reactions were as follows: 1.5 minutes at 95°C, 1.0 minute at 55°C, and 2.5 minutes at 72°C (Palumbi, 1990). The reactant volume was 25ul, and the reactants and their final concentrations were: 1x reaction buffer; 0.800mM dNTP mix (0.200mM each), 0.6uM each primer, 2 Units *Taq* (BRL), and 4 mM MgCl2. The PCR products from the D-loop/cytB and ND5/6 amplification reactions, approximately a total of 4.6 KB of 16.5 KB total genome size, were combined for enzymatic digestion.

Composite haplotypes were digested with twelve tetrameric restriction enzymes, *Alu*I, *Bfa*I, *Bst*U1, *Dde*I, *Dpn*II, *Hae*III, *Hha*I, *Hin*fI, *Msp*I, *Nla*III, *Rsa*I, and *Taq*I, one multipentameric enzyme, *Ava*II, and one multihexameric enzyme, *Hinc*II (New England Biolabs, NEB). Diagnostic samples were digested with two enzymes, either *Alu*I and *Dpn*II or *Alu*I and *Hae*III. Digested products were visualized on 2% agarose gels stained with ethidium bromide, and restriction sites were inferred from the fragment patterns.

Sequencing analysis

We sequenced 468bp of the ~2kb ND1 region for the purpose of further resolving relationships among mitochondrial haplotypes. However, as we were unsuccessful at sequencing all RFLP haplotypes, only 24 of the 32 haplotypes were included in the combined RFLP and sequencing phylogenetic analysis (App. 3). The remaining RFLP haplotypes were not particularly divergent (they each differed by only a single mutation from one of the haplotypes sequenced) and therefore we felt their absence was unlikely to strongly affect the phylogenetic resolution of major clades. PCR conditions and reagent concentrations for sequencing reactions were done as described in Redenbach and Taylor (1999). Primers NDB and NDC (Redenbach and Taylor, 1999) were used for the ND1 amplification.

Purification of PCR products was done using the Qiagen Qiaquick PCR Purification kit, with termination PCR reactions conducted according to Redenbach and Taylor (1999). Centri-Sep purification columns were used to remove extra ddNTPs and primers, after which the PCR products were sequenced by an ABI 377 automated sequencer. The programs *Pcgene* and *Clustal* (Bairoch, 1991) were used to align and format sequences.

Phylogenetic analysis

Individual enzymatic digestion patterns and composite haplotype identities were compiled into a presence-absence matrix of restriction sites using *Generate* from the REAP software package (McElroy *et al.*, 1992). A weighted estimate of evolutionary divergence from different restriction enzyme fragment patterns was computed using the program *D* (as per Nei and Tajima 1981; Nei and Tajima, 1983; Nei and Miller, 1990, eq.4). For phylogenetic analysis among haplotypes, ND1 sequence data were converted to binary data and combined with composite RFLP data. Genetic matrices included 142 RFLP characters and 117 of the 468 sequence characters. All variable nucleotides were included. Matrices were bootstrapped 100 times in *Seqboot* and run through Mix (Felsenstein, 1993) for parsimony analysis, and Boot (Jaarola and Tegelstrom, 1995) and Neighbor (using neighbor-joining; Felsenstein, 1993)) for distance analysis. Consense was used to find the majority-rule consensus trees for both methods, and midpoint rooting was done in Retree. Midpoint rooting, which places the root halfway between the two most divergent haplotypes, was done because using cutthroat trout as an outgroup proved too difficult with restriction site data. Drawgram was used to construct all final phenograms.

Genetic diversity

Diversity indices were determined using *DA* from the REAP package (McElroy *et al.*, 1992). *DA* estimates haplotype and nucleotide diversity within populations and computes nucleotide divergence between pairs of populations. Haplotype diversity measures the probability that any two individuals in a sample are different, and is calculated according to Nei (1987, equations 8.4, 8.5 and 8.12). Nucleotide diversity is a composite statistic based on both the number of different haplotypes and the genetic divergence among them. It calculates diversity as per Nei and Tajima (1981, Nei, 1987, eqs. 10.19, 10.7). Diversity analyses were important for identifying potential refugia as former refugia would likely be more diverse than recently colonized sites.

Relationships among Regions

Genetic similarity among watersheds in rainbow trout was determined using the Holsinger and Mason-Gamer *Nucleodiv* program (Holsinger and Mason-Gamer, 1996). *Nucleodiv* uses an Fst analog, *g*st, to perform a cluster analysis and computes the divergence among groups based on the probability that any two haplotypes, chosen at random from two different populations, match (Holsinger and Mason-Gamer, 1996).

In addition to the *Nucleodiv* program, *Monte* was used from the REAP software package (Roff and Bentzen, 1989, McElroy *et al.*, 1992) which uses Monte Carlo simulations to test for geographic heterogeneity. In the *Monte* analysis, repeated randomizations (1000 times) of the data were done to determine the probability of getting the χ^2 value obtained simply by chance. The significance of all nodes in the *Nucleodiv* tree were tested by collapsing all populations stemming to the right of a given population into one group and comparing that group to the other population at that node.

Partitioning of variation within and among populations and regional groups was then assessed using an Analysis of Molecular Variance, or AMOVA (Schneider *et al.*, 1997). An

AMOVA computes three statistics: the proportion of variation among groups (Φct), the proportion of variation among populations within groups (Φsc), and the proportion of variation within populations (1-Φst) (Excoffier *et al.*, 1992). Diversity was based on both frequency differences and interhaplotypic distances. We partitioned biogeographic regions in different ways to determine which grouping accounted for the most genetic variation. We first ran AMOVA analyses using watersheds as groups (see *Watershed*, Table 3) and then imposed an additional hierarchical level to assess whether or not regional structure could be detected and, if so, which regional structure accounted for the most variation. The three regional groupings used were as follows: i.) grouping "coastal" watersheds together and "redband" watersheds together according to Behnke's (1992) taxonomic designations (see *Behnke*, Table 3), ii.) grouping watersheds according to the two main groups resulting from the *Nucleodiv* analysis (see *Nucleodiv*, Table 3) and iii.) grouping those watersheds associated with particular glacial refugia together and those watersheds associated with postglacial recolonization together (see *Refugia*, Table 3).

RESULTS

Genetic Variation and Relationships Among mtDNA Haplotypes

Approximately 123 restriction sites corresponding to a total of 504 base pairs were analyzed with recognition enzymes. Overall, 32 RFLP composite haplotypes were found (App. 2). The most common haplotype, haplotype 1, was found in every major watershed with an overall frequency of 45%. Haplotypes 24 and 31 were also frequently found, comprising 12.6% and 11% of the samples, respectively. Twenty-four of the haplotypes were found in five (<2%) or fewer samples (Fig. 3).

The ND1 region was sequenced in 24 of the RFLP haplotypes (App. 3). Sequencing analysis indicated seven haplotypes (RB1-7), with 26 of the 32 mutations occurring strictly between rainbow and cutthroat trout (App. 4). The overall transition to transversion ratio in ND1 was 26:6 (about 4:1), with transversions occurring only between rainbow and cutthroat trout sequences.

The twenty-four sequence haplotypes were analyzed phylogenetically using both RFLP and ND1 sequencing data. Bootstrap support in the parsimony analysis was 99% for clade B and 71% for clade A. For distance analysis, bootstrap support was 96% for clade B and 73% for clade A (Fig. 4). Sequence divergence among haplotypes ranged from 0.1- 1.75% based on RFLP data alone, with an average of 1.03%. Using a molecular clock of approximately 1% divergence per million years (Smith, 1992), the average sequence divergence between the clades corresponded to a divergence time of approximately 1.03 million years ago, i.e. before the last glaciation.

The diagnostic analysis distinguished the two clades from each other and was done to better resolve the geographic distributions of the two clades. Based on both composite and diagnostic analyses, clade A was found in every major watershed except the upper Liard, while clade B was mainly restricted to coastal watersheds. Clade B was absent in the Columbia, the Snake, the Athabasca, and Kamchatka, and was found in only two samples of 25 from Alaska (Table 1, Figs. 5, 6).

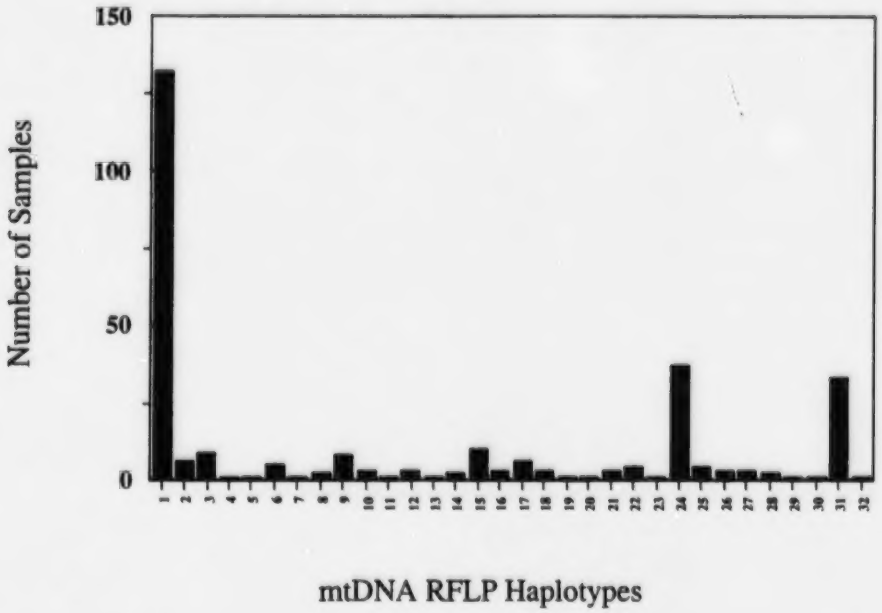

Figure 3. The number of each composite haplotype found in the total sample of 292 composite haplotypes of *O. mykiss* mtDNA.

Genetic Diversity

We grouped sample sites into their respective watersheds, where possible, to assess diversity on a regional scale (Table 2). Haplotype diversity was relatively high in many watersheds, but it was particularly high in several regions hypothesized to have been potential refugia in the last glaciation, including the Columbia and Snake rivers, Alaska/ Kamchatka and the Queen Charlotte Islands (Fig. 7) (McPhail and Lindsey, 1986). Interestingly, this high level of haplotype diversity was often accompanied by low nucleotide diversity, indicating the presence of many haplotypes that were closely related to each another.

In contrast, four watersheds had lower haplotype diversity than the rest (Fig. 7): the upper Fraser, Peace, and Athabasca rivers, and the North Coast/ Yukon region, as expected for recently colonized regions. In these watersheds, nucleotide diversity was relatively high, indicating few haplotypes with high divergence among them. These watersheds were all glaciated until 15-10,000 years ago and, given lower nucleotide diversity at putative refugia, this pattern is consistent with recolonization from more than one refuge.

Table 1. Clade A and Clade B composite and diagnostic haplotypes. Composite haplotypes were digested with 14 restriction enzymes whereas diagnostic haplotypes were digested with 2 enzymes to determine clade identity. Sites without asterisks were analyzed for a composite haplotype; sites with asterisks were analyzed for diagnostic haplotypes. These site groupings correspond with Figures 5 and 6.

Regions (Sites)	*A*	*B*	*Regions (Sites)*	*A*	*B*
OREGON	7		LOWER FRASER	13	3
Alsea R.	7		Anderson R.	2	
			Emory Ck.*	1	3
PUGET SOUND	9	11	American Ck.*	5	
Tahuya R.	3	2	Bridge R.*	5	
Bogachiel R.*		4			
Nooksak R.*	1	3	MIDDLE FRASER	35	1
Deer Ck*	3	2	Fish L.	6	
Tucannon R.*	1		Tzenzaicut L.	5	
Lewis R.*	1		Puntzi Ck.*	5	
			Euchiniko r.*	4	1
N. VANCOUVER ISLAND	6	10	Chilko/ Chilcotin R.*	5	
Keogh R.	1	4	Gaspard Ck.*	4	
Mahatta R.	3	2	Baezaeko*	4	
Klootchlimmis Ck.	2	4	Coglistiko*	2	
S. VANCOUVER ISLAND	13	20	NICOLA/ THOMPSON	18	6
Cowichan R.	3	2	Pennask L.	5	
Lucky Ck.	1	4	Bonaparte R.	2	3
Elsie L.		5	Coldwater R.*	4	1
Bamfield		1	Deadman R.*	4	
Robertson Ck	3	2	Spius Ck.*	3	2
Quinsam R.	4	1			
Junior L.*		3	NECHAKO RIVER	6	6
Efflingham R.*	2	2	Cheslatta L.	4	1
			Tahtsa R. *		5
LOWER MAINLAND	10	11	Brewer's Ck.*	2	
Chehalis R.	2	1			
Capilano R.	1	4	UPPER FRASER	16	6
Vedder R.	1	4	Bivouac Ck.	5	
Squamish R.*	1	2	Dome Ck.	5	
Abbotsford*	5		Baptiste Ck.*	4	
			Forfar*	2	1
CENTRAL COAST	7	8	Hautete Ck.*		5
Dean R.	4	1			
Ashlulm Ck.*	2	3	SKEENA RIVER	28	11
Abuntlet L.*	1	4	Babine R.	3	2
			Morice R.	3	2
KITIMAT	11	2	Canyon Ck.	3	2
Kitimat R.	3	2	Goathorn Ck.	4	1
Union L.*	5		Toboggan Ck.	3	2
Khtada L.*	3		Kispiox R.*	4	1
			Sustut R.*	4	1
			Natlan Ck.*	4	

(Table 1, cont'd.)

Regions (Sites)	*A*	*B*
QUEEN CHARLOTTES	4	19
Honna R.		5
BVM	2	3
Brent Ck.*	2	
BVM*		3
Lower Phantom Ck.*		8
NORTH COAST	17	8
Tahltan R.	4	2
Taku R.	4	2
Disella L.*	2	3
Iskut R.*	5	
Taku R.*	2	1
UPPER LIARD	0	11
Kutcho Ck.		5
Wolverine*		3
Blue Sheep L.*		3
YUKON	10	0
McIntyre	5	
Kathleen R.	5	
ALASKA	23	2
Karluk R.	7	
Natnek R.	6	
Nushagak R.*	3	2
Neguthluk R.*	7	
KAMCHATKA	11	0
Kavachina	5	
Snotolvayam	6	
OKANAGAN	7	0
Goatskin Ck.	2	
Goatskin Ck.*	3	
Fall Ck.*	2	

Regions (Sites)	*A*	*B*
NASS RIVER	5	10
Meziadin R.		5
Paw L.*		5
Niska Ck.*	5	
WILLISTON RESERVOIR	9	12
Firesteel R.		3
Mesilinka R.	3	3
Nation R.	5	
Ingenika R.*	1	1
El Condor*		3
Firesteel R.*		2
PEACE RIVER	20	5
Chowade R.	5	
Graham R.	5	
Kiskatinaw R.		5
Moose L.	5	
Burnt R.*	1	
Sekunka R.*	1	
Elizabeth Ck.*	3	
ATHABASCA RIVER	15	0
Wampus Ck.	5	
Halpeny Ck.	5	
Cabin Ck.*	5	
KOOTENAY LAKE	23	0
Bayonne Ck.	4	
Lardeau R.	5	
Procter Ck.	5	
Fry Ck.*	4	
Summit Ck.*	5	
ARROW LAKE	12	0
Halfway R.	5	
Hill Ck	5	
Tumtum Ck.	2	
SNAKE RIVER	14	0
Dworshak R.	7	
Selway R.	7	
Total (all samples)	349	162

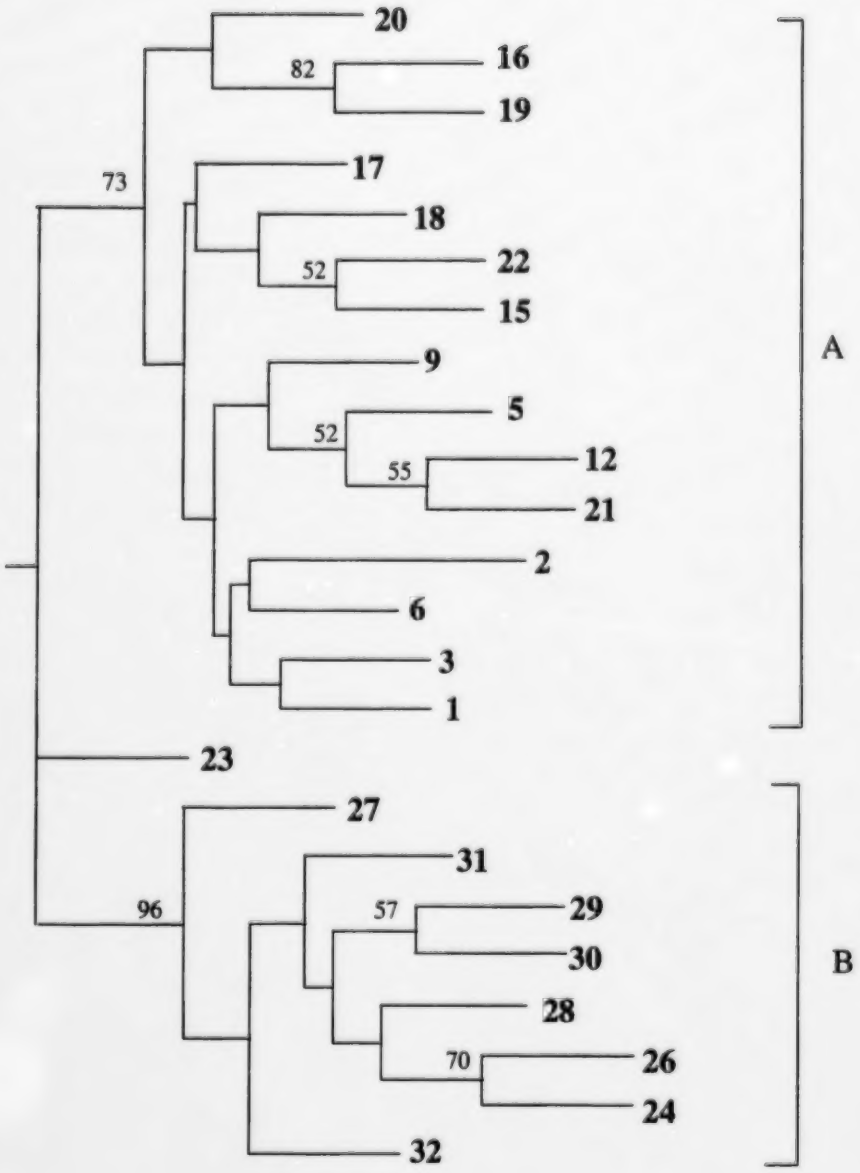

Figure 4. Midpoint-rooted consensus phylogeny of combined data from mtDNA RFLP analysis and ND1 sequencing using *Boot* and neighbor-joining in *Neighbor*. Circles indicate those haplotypes also sequenced for the right domain of the D-Loop region. Bootstrap values above 50% are reported. Haplotypes belonging to clade A and clade B are indicated on the right.

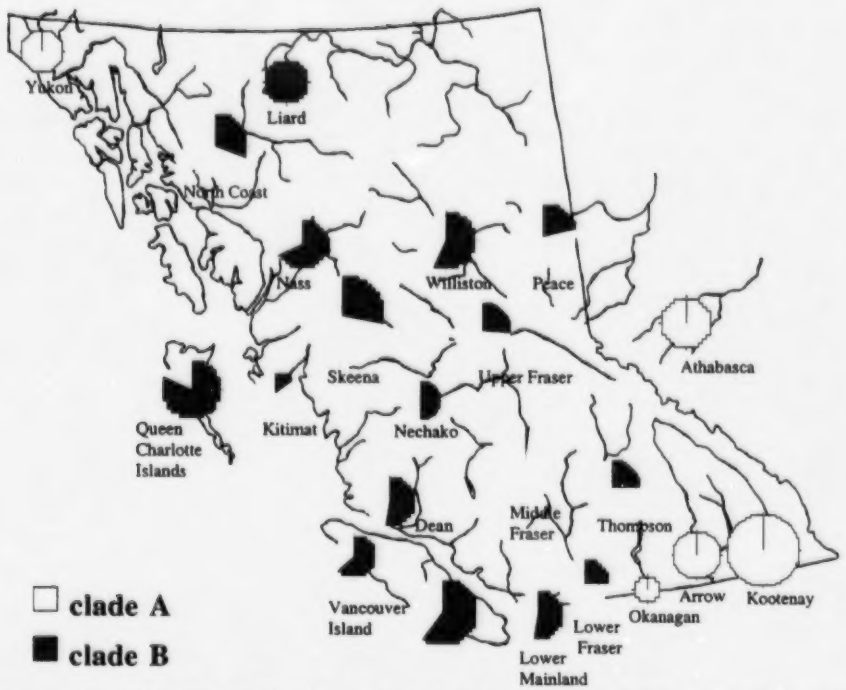

Figure 5. Distributions of mtDNA RFLP clade A and clade B haplotypes in *Oncorhynchus mykiss* in British Columbia. The area of the pie corresponds to the relative number of both composite and diagnostic samples taken, with the smallest sample (Okanagan) equaling seven individuals.

Figure 6. Distributions of mtDNA RFLP clade A and clade B haplotypes in *Oncorhynchus mykiss* in the North Pacific. The area of the pie corresponds to the relative number of both composite and diagnostic samples taken, with the smallest pie (Oregon) equaling 7 individuals.

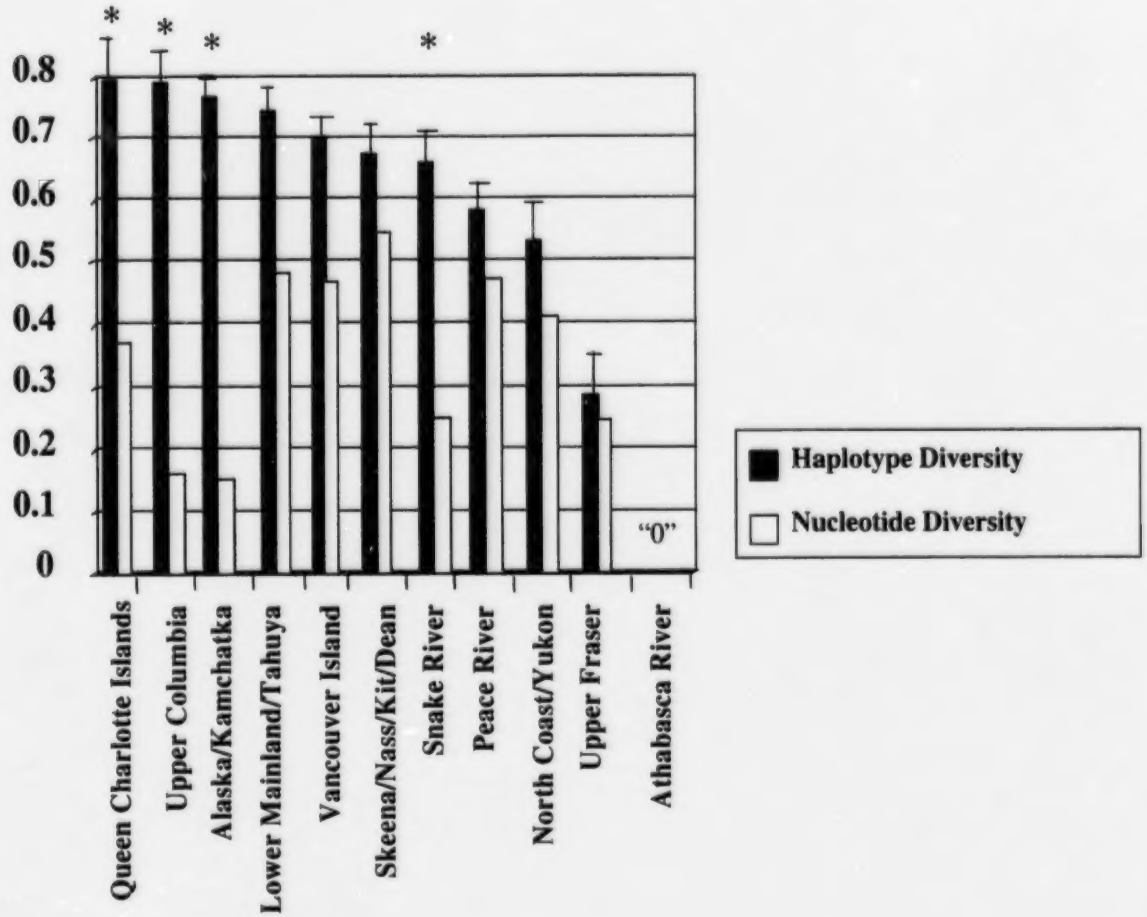

Figure 7. Diversity measures of *O. mykiss* mtDNA RFLP haplotypes in order of largest to smallest in haplotype diversity. Diversity was measured in watersheds throughout the region sampled using the program *D* from REAP (McElroy *et al.*, 1992). Haplotypic diversity is a probability measure reflecting the number of different haplotypes in a region (with standard error bars represented) whereas nucleotide diversity is a composite measure reflecting both number of different haplotypes and divergence among haplotypes. Asterisks signify putative refugia (McPhail and Carveth, 1992).

Table 2. Composite haplotypes of *O. mykiss* mtDNA found within watershed groups.

	VI	LM	FRA	QCI	SK	NCY	PEA	ATH	COL	SNA	A/K	ORE	OK
1	9	3	32	2	17	17	19	10	10		8	3	2
2	3	3											
3	2										7		
4					1								
5					1								
6											4	1	
7						1							
8											2		
9			2				1			5			
10												3	
11	1												
12											3		
13							1						
14							2						
15		1							3	6			
16	1								2				
17					3				3				
18									3				
19									1				
20	1												
21										3			
22									4				
23					1								
24	21	8		1		8							
25	2	2											
26				3									
27				3									
28					2								
29				1									
30			1										
31	1	1	3		15	1	11						
32	1												
T	42	18	38	10	40	27	34	10	26	14	24	7	2

VI = Vancouver Island
LM= Lower Mainland
FRA= Fraser River
QCI= Queen Charlotte Islands
SK= Skeena, Kitimat, Nass, Dean rivers
NCY= Tahltan, Taku, Stikine, Tatshenshini
PEA= Peace River
ATH= Athabasca River
COL= upper Columbia River
SNA= Snake River
A/K= Alaska/ Kamchatka
ORE= Oregon
OK= Okanagan

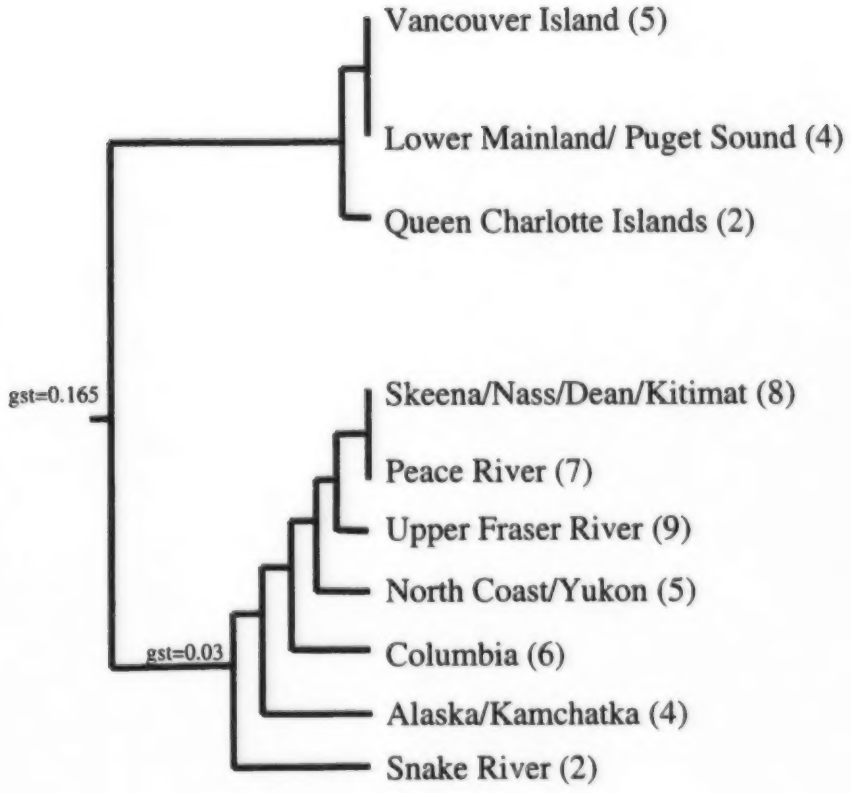

Figure 8. Population cluster analysis among watersheds using *Nucleodiv* (Holsinger and Mason-Gamer, 1996). Positive g_{st} values, indicated at two nodes, were significant at $p<0.01$. Branching patterns at other nodes represent the *Monte Carlo* simulation analyses (using *Monte* by Roff and Bentzen, 1989). The "0" branch lengths at the Vancouver Island/ Lower Mainland node and the Peace River/ Skeena River node indicated they were not significantly different from one another. The number of localities sampled in each watershed group is indicated in parentheses.

Relationships among Regions

Population cluster analysis was based on the population units defined in Table 2. The population cluster analysis suggested that the major geographic division was between two groups: the Queen Charlotte Islands, Vancouver Island and Lower Mainland in one, and the Columbia, Fraser, Peace, Skeena, Taku, and Stikine rivers in the other (Fig. 8). Although only two branches resulted in positive *g*st values, *Monte* resulted in significant differences at all nodes with the exceptions of the Vancouver Island/ Lower Mainland node and the Peace/ Skeena node (see Fig. 8).

All AMOVA analyses resulted in significant structure (see Table 3). Regional genetic structure was shown in the province with all three groups (*Behnke, Nucleodiv,* and *Refugia*) in that the among-region variation (Φct) was larger than among watersheds within regions (Φsc), and the two together accounted for a larger percentage of variation than did among watersheds without a regional component (*Watersheds*). Despite the overall similarity in the proportions of variation among *Behnke, Nucleodiv,* and *Refugia,* however, *Refugia* stood out as minimizing variation among watersheds relative to among regions, for accounting for the highest among-group variation (Φct), and for being the most highly significant of the three. Furthermore, after adjusting for multiple tests with a sequential Bonferroni correction, *Refugia* was the only test that accounted for significant regional structure.

Table 3. Results of AMOVA analysis in which the range of *O. mykiss* within British Columbia was divided in four ways (see below). An AMOVA was performed on each of these, calculating Φct, the proportion of variation among groups; Φsc, among populations within groups; and 1-Φst, the proportion of variation within populations. After adjusting for multiple tests with a Bonferroni correction, significant p-values are indicated on the far right (S=significant, NS= not significant).

Structure	Hierarchical Levels	% Variation	P-value	
Watersheds	Among watersheds	16.81 %	P=0.00	S
	Among populations within watersheds	26.23 %	P=0.00	
	Within populations	56.95 %	P=0.00	
Nucleodiv	Among watersheds	20.69 %	P=0.02	NS
	Among populations within watersheds	10.00 %	P=0.00	
	Within populations	69.32 %	P=0.00	
Behnke	Among watersheds	19.45 %	P=0.02	NS
	Among populations within watersheds	8.88 %	P=0.00	
	Within populations	71.67 %	P=0.00	
Refugia	Among watersheds	22.01 %	P=0.00	S
	Among populations within watersheds	5.77 %	P=0.00	
	Within populations	72.22 %	P=0.00	

Watersheds: (10 groups) (n=260)
Vancouver Island: Keogh, Cowichan, Quinsam, Somass, Lucky, Elsie, Bamfield, Klootchlimmis, Mahatta (n=42);
QCI: Honna, BVM (n=10);
Lower Mainland/ Tahuya: Chehalis, Capilano, Vedder, Tahuya (n=18)
upper Fraser: Anderson, Fish, Tzenzaicut, Pennask, Bonaparte, Cheslatta, Bivouac, Dome (n=39)
Skeena/Nass/Kitimat/Dean: Morice, Babine, Canyon, Goathorn, Toboggan, Meziadin, Kitimat, Dean (n=40)
North Coast/Yukon: Tahltan, Taku, Kutcho, McIntyre, Kathleen (n=27)
Peace/Williston: Chowade, Graham, Kiskatinaw, Moose, Firesteel, Mesilinka, Nation (n=34);
Athabasca: Wampus, Halpenny (n=10)
Columbia: Halfway, Hill, Tum Tum, Procter, Bayonne, Lardeau (n=26)
Snake: Dworshak, Selway (n=14)

Nucleodiv: (2 groups)
Coastal: Queen Charlotte Islands, Vancouver Island, Lower Mainland/ Tahuya (n=70)
Inland: Skeena/Nass/Kitimat/Dean, North Coast/Yukon, upper Fraser, Peace/Williston, Athabasca, Columbia, Snake (n=190)

Behnke: (2 groups)
Coastal: Lower Mainland/Tahuya, Vancouver Island, QCI, Skeena/Nass/Kitimat/Dean, North Coast/ Yukon (137)
Inland: upper Fraser, Peace/Williston, Athabasca, Columbia, Snake (n=123)

Refugia: (3 groups)
Coastal (refuge): Lower Mainland/Tahuya, Vancouver Island, Queen Charlotte Islands (n=70)
Interior (recolonized): Skeena/Kitimat/Nass/Dean, North Coast/Yukon, upper Fraser, Peace/Williston, Athabasca (n=150)
Inland (refuge): Columbia, Snake (n=40)

DISCUSSION

Sources of Information on Phylogenetic Relationships

There are two main sources of information on the phylogenetic relationships among populations within a species. Glacial and geological history provides clues as to the locations of potential refugia and colonization routes and various molecular markers, that are believed to be selectively neutral, can be used to infer ancestral relationships. Adaptive variation seems to be mainly a response to local environmental conditions and provides little information on historical relationships among populations.

Molecular markers provide evidence of ancestral relationships in three forms: gene frequency variation among populations, within-population diversity, and estimates of relationships among alleles or haplotypes. Similarity in gene frequencies among populations indicates either common ancestry or chance similarity. Chance similarity can be ruled out by either repeated results from different markers (different isozymes, mtDNA etc.) or by repeated differences/similarities among distant/adjacent populations. The consistent difference in allozyme frequencies between coastal and interior populations of rainbow trout represents a typical use of this type of information (Utter *et al.* 1980). These differences suggest that coastal populations of rainbow trout share a common ancestry (phylogeny) that differs from that of interior populations.

In addition to various measures of genetic differentiation among populations, molecular markers can also provide estimates of the amount of neutral genetic diversity within populations. Source populations are thought to have higher diversity than recently colonized areas due to the successful colonization of only a subset of the haplotypes from any particular source area (Templeton *et al.*, 1995; Hewitt, 1996; Weider and Hobaek, 1997). Empirical evidence has repeatedly shown that high diversity is associated with formerly ice-free areas and low diversity with recolonized areas (e.g. Cann *et al.*, 1987; Jaarola and Tegelstrom, 1995; Templeton *et al.*, 1995; Hewitt, 1996; Merila et al. 1997; Soltis *et al.*, 1997; Weider and Hobaek, 1997).

DNA markers also provide information on the relationships among alleles from which a population's history can be inferred. Populations that have been isolated for a long period of time or that were recolonized from a single source population are generally characterized by closely related haplotypes (Avise, 1994), whereas those that were colonized by multiple sources may have distantly related haplotypes present.

Previous Evidence of Phylogenetic Relationships in Rainbow Trout

The geological evidence suggests that B.C. was recolonized by rainbow trout that survived the last glaciation in ice-free refugia that were isolated from one another for substantial periods of time. Virtually all of B.C. was under a thick layer ice from about 60,000-15,000 years BP (Fig. 9). Ice-free refugia for freshwater fish were clearly present to both the north and south. In addition, small areas of the coast appear to have remained ice-free. All of these represent potential refugia for rainbow trout, but it is not clear from the geological evidence that the smaller refugia were capable of supporting freshwater fish species. Among the major refuge

areas in the south, there is general agreement that rainbow trout took refuge west of the Rocky Mountains (McPhail and Lindsey 1986) but may also have been east of the Rockies (Carl *et al.* 1994).

Extensive older data on isozyme allele frequencies are available for rainbow trout. This data suggests that there were two separate refugia in the south divided by a line running along the Cascade mountain crest. This subdivision extends into BC (Parkinson 1984) with Alaskan and Kamchatkan populations falling into the coastal group (Utter *et al* 1980). By contrast, Skeena River populations did not fit well into either of these groups (Parkinson, 1984). The lack of comparative allozyme data for rainbow trout from Queen Charlotte Islands and many other areas in northern BC, however, limits the interpretation of allozyme data from the Skeena watershed.

In the following summary, we utilize molecular data from Taylor (1995), Carl *et al.* (1994), Okazaki (1984), Parkinson (1984), Utter *et al.* (1980), Reisenbichler *et al.* (1992), Reisenbichler and Phelps (1989) and Allendorf (1975) as well as zoogeographic information from McPhail and Lindsey (1986) to formulate a hypothesis concerning the origin and phylogenetic structure of rainbow trout populations in BC. We believe that this hypothesis represents the simplest explanation of the current data on the geographic distribution of genetic diversity in rainbow trout. Although assertions of specific differences among populations have been examined statistically, statistical tests of the overall hypothesis is difficult because of the non-overlapping nature of the various data sets. We have built our work on previous hypotheses and expect that our hypothesis may be modified in the future. Nevertheless we believe that our hypothesis, as outlined below, forms the best scientific basis for the conservation of phylogenetic groups in rainbow trout. We will first identify source areas, then potential recolonization pathways, and the implications for conservation.

Hypothesized Source Areas for British Columbia

The most diverse and, presumably, the ultimate source populations of rainbow trout is in California (Behnke, 1992, Nielsen *et al.*, 1994; McCusker, 1999). The available evidence suggests that rainbow trout did not colonize B.C. directly from this source but rather invaded from three refugia (Fig. 9). Of these, only the north coast is inside B.C. with the others to the south. These southern refugia, however, were in close proximity to southern B.C. and this area probably provides a good representation of the diversity of these source populations. Populations in other areas seem to represent a subset of the genetic material from these three refugia. In addition, many areas seem to represent contact zones with genetic material contributed from more than one refuge.

Much of the lower Columbia Drainage is thought to have been ice-free during the last glaciation and an important refuge for most freshwater-dependent species in British Columbia (McPhail and Lindsey, 1986). The Columbia drainage has high haplotype diversity, which is the signature of a source area. High diversity within clade A in the Columbia drainage (Fig. 7) populations suggests that this was a likely source for many clade A haplotypes in the British Columbia interior. Isozyme data confirms a strong coastal vs. interior split in the unglaciated U.S. as well as B.C. (Parkinson 1984, Utter et al. 1980). Other species, such as bull trout,

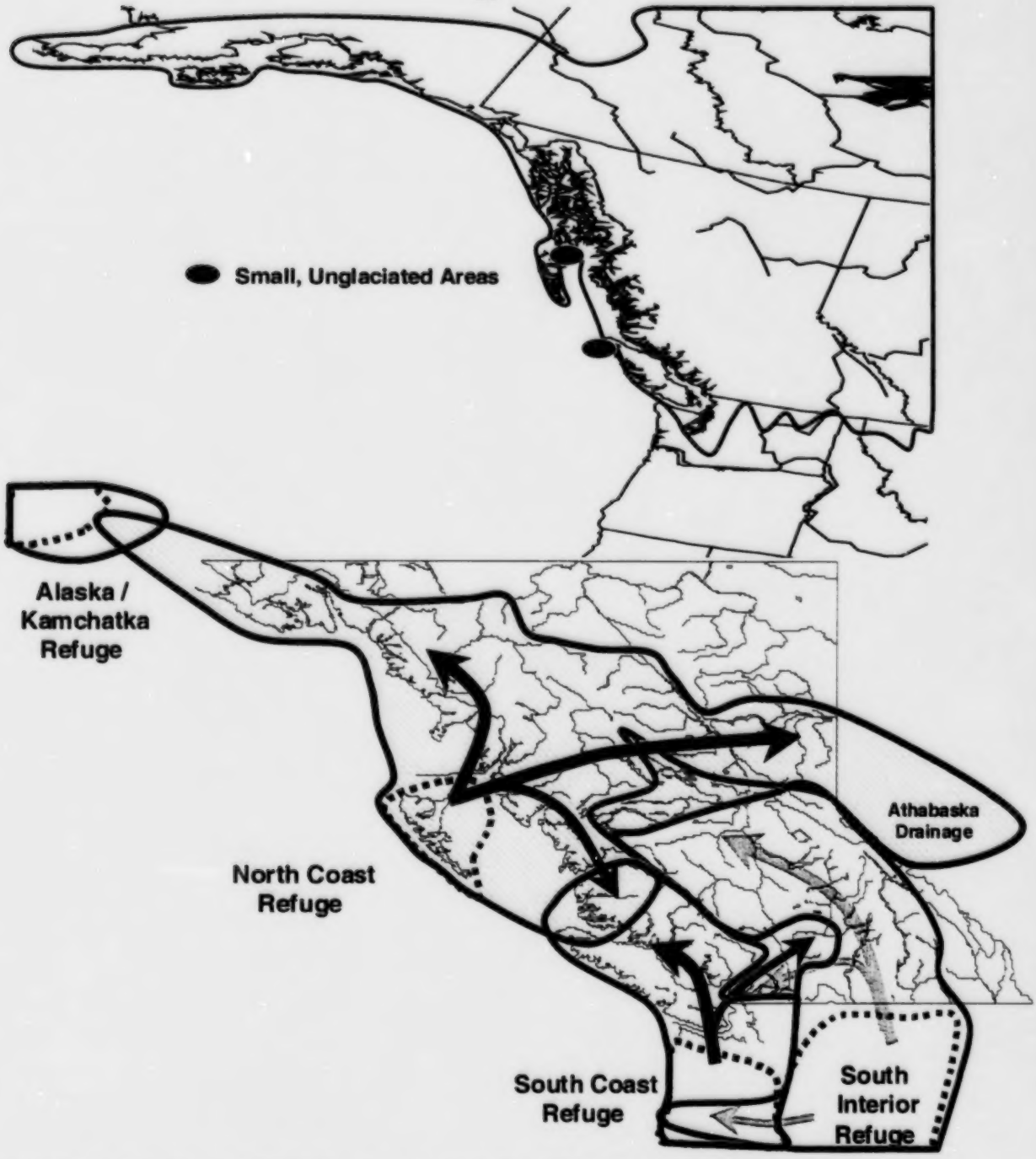

Figure 9. Approximate maximum extent of ice cover during the last glaciation (heavy black line, top panel) and locations of refugia hypothesized in text (bottom panel). Approximate location of each refuge is enclosed with a dotted line while the solid line enclosed the hypothesized dispersal of genetic material from each refuge. Arrows illustrate hypothesized dispersal routes. The North Coast Refuge is located near the location of the Queen Charlotte Islands. Areas colonized from the Alaska/Kamchatka refuge are presumed to be outside of B.C. The Athabaska Drainage is also outside of BC but is not assumed to be a separate refuge.

Salvelinus confluentus, also show evidence of having survived glaciation in the Columbia River, and expanded throughout most of B.C. via an interior colonization route (Taylor *et al.*, 1999).

Rainbow trout almost certainly survived the last glaciation in coastal areas to the south of B.C. since the Wisconsin ice sheet extended only as far as Olympia, Washington. Molecular variants in adjacent streams are typical of coastal populations. Relatively high levels of diversity in both mtDNA clades (Fig. 7) on Vancouver Island and the Lower Mainland indicate either that a glacial refuge was located nearby or that this region was recolonized by large populations, or both. Brooks Peninsula on Vancouver Island was also unglaciated. Although various plants probably survived in this refuge, it probably did not support fish (B.C. Museum Publication). The Chehalis River south of the Olympic Peninsula is thought to have been used by rainbow trout as a glacial refuge due to their distinct chromosome numbers in this region (Thorgaard, 1983). Bull trout (Taylor *et al.*, 1999) and several other species (McPhail and Carveth 1992) also appear to have invaded southwestern B.C. from a Chehalis refuge. However, recolonization from more southerly areas of the Pacific Coast by anadromous steelhead is also possible. Allozyme frequencies indicate that rainbow trout from Puget Sound, the Lower Mainland, and Vancouver Island are typical of coastal trout (Parkinson, 1984), and minisatellite data corroborate that this region belongs to a coastal group (Taylor, 1995).

Evidence exists for a B.C. North Coast refuge in several species in and around the Queen Charlotte Islands. Wood *et al.* (1994) showed that a coastal refuge was probably used by sockeye salmon, *O. nerka*. Evidence from several terrestrial vertebrates, including the song sparrow, black bear, pine marten and weasel (Byun *et al.*, 1997), and plant species (Ogilvie, 1989) suggests ice-free regions in the Brooks Peninsula and the Queen Charlotte Islands were used as refugia in the last glaciation. Again the high haplotype diversity is indicative of a source area near the Queen Charlotte Islands which may have been below the current sea level (Figure 9). The common alleles from the mtDNA differ between the North and South coast (Table 2) suggesting that north and south coast populations originated in different refugia. No isozyme samples are available from the Queen Charlotte Islands, but isozyme frequencies in the nearby Skeena River are not representative of either South Coast or South Interior populations (Parkinson 1984). To the north, Alaskan and Kamchatkan populations lack mtDNA B haplotypes and also differ from north coast populations at key isozyme loci.

The region known as Beringia was another potential refuge for rainbow trout during the last glaciation and the high mtDNA diversity in rainbow trout samples from Alaska and Kamchatka suggests that rainbow trout may have used this northern refuge (Fig. 7). In contrast to coastal populations from B.C., however, the mtDNA B clade was virtually absent from Alaskan and Kamchatkan samples. Furthermore, clade A haplotypes present in Alaska and Kamchatka (haplotypes 1, 3, 6, and 12) were not observed in the northwestern corner of British Columbia (haplotypes 1, 7), which suggests that B.C. was not colonized by trout from Beringia.

Okazaki (1984) found that maturing steelhead from the western Pacific lacked the low levels of LDH variation found in south coastal populations and they suggested the presence of a Beringian refuge. Other salmonid species, including chum salmon, (*O. keta*), sockeye salmon (*O. nerka*), pink salmon (*O. gorbuscha*), and chinook salmon (*O. tshawytscha*) also exhibit

genetic divergences between Alaskan and more southern populations (Cronin *et al.*, 1993; Varnavskaya and Beacham, 1992; Taylor et al., 1996; Seeb and Crane, 1999). Lindsey and McPhail (1986) suggested that rainbow trout likely recolonized the Alsek River in the Yukon from the Gulf of Alaska, but they imply that recolonization did not extend much further south.

The available evidence suggests that source populations in other refugia, such as the Great Plains, is unlikely. McPhail and Lindsay (1986) suggest that rainbow trout were not present in refugia east of the Rockies during the last glacial. On the basis of allozyme data, Carl *et al.* (1994) argue that rainbow trout from the Athabasca drainage in Alberta represent a phylogenetic lineage that is independent of other rainbow trout. However, mtDNA diversity indices are low and the only mtDNA haplotype present is common in other populations in B.C. In addition, McCusker (1999) points out that the some isozyme alleles found to be unique to this population (Carl *et al.*, 1994) are shared with coastal cutthroat trout. We consider this drainage to be a separate phylogenetic group, which may have originated through hybridization with cutthroat rather than long-term isolation in a refuge east of the Rocky Mountains, but this remains a preliminary hypothesis at present.

Source Populations for Recolonized Areas

Virtually all B.C. populations represent recolonized rather than source areas. Evidence from other species (e.g. Bernatchez, 1995) and previous evidence from rainbow trout suggest that recolonized areas often consist of populations derived from a single refuge separated by sharp geographical boundaries from populations derived from alternative refugia. The data for B.C. rainbow trout suggest that boundaries between phylogenetic groups are sharp in some locations but are blurred by contributions from more than one refuge in others. In most cases, the location and extent of blurred boundaries are poorly defined by the data, nevertheless we have been able to define many areas that are likely to support a single phylogenetic group as well as which areas are likely to represent mixtures.

Most of the Fraser and Columbia river basins appear to have been colonized by fish from a southern interior (Columbia) refuge. Allozyme frequencies in the Fraser are similar to the Columbia and areas father south and the mtDNA B haplotype is missing from the Snake, Columbia, Middle Fraser and Athabasca drainages. The presence of the B haplotype and noticeably higher LDH-100 frequencies in Thompson River steelhead suggest that this drainage also received some genetic material from the South Coast. The presence of the B mtDNA haplotype in the northern reaches of the Fraser, particularly in the Nechako, suggest that some fish from a coastal refuge have entered this area perhaps via the Skeena Drainage. Some introgression with coastal populations is also suggested by allozyme data (Parkinson, 1984) where LDH-100 frequencies from the lower Fraser are lower than other coastal populations and LDH-100 frequencies in Thompson River steelhead are higher than in other interior populations.

The composite haplotypes in the B clade in Vancouver Island and the south coast (mainly haplotype 24) differ from those in the north interior of the province (mainly haplotype 31), although the upper Liard River in the north is an exception (all haplotype 24). Allozyme and minisatellite frequencies also differ between the Skeena River and those further south

(Vancouver Island, Dean River) (Parkinson, 1984; Taylor 1995). Fish from the South Coast refuge presumably colonized drainages in Puget Sound, the Lower Fraser, and Vancouver Island.

The northern half of the Province, including the Peace drainage, appears to have been colonized from a refuge around the Queen Charlotte Islands given the high frequency of haplotype 31, particularly in the Skeena and upper Peace rivers. Haplotype 31 is unlikely to have come from the Columbia or Beringia due to the absence or very low frequency of clade B haplotypes in these regions. Haplotype 31 was not found among Queen Charlotte Island samples. Though the composite haplotype sample is very small (10) and diverse (5 types), haplotype 31 was not found in a diagnostic analysis of 12 samples (10 clade B, 2 clade A). However, the Queen Charlotte Islands and Hecate Strait is still considered a source region for the Skeena and Peace rivers due to the frequency of the clade B haplotypes in these rivers, albeit not one currently found in our sample from the Queen Charlottes. We hypothesize that haplotype composition in this refuge may have been slightly different at the time of recolonization. Allozyme frequencies in the Skeena also differ from those in Vancouver Island (Parkinson, 1984), supporting that the area was mainly derived from a northern refuge. In addition, minisatellite alleles that are present in relatively high frequency (up to 10%) on Vancouver Island are missing from the Skeena drainage (Taylor 1995).

The north coast (Queen Charlotte) refuge hypothesis is not supported by the Haplotype 1 frequency data. Haplotype 1 is found at high frequencies in the Fraser Drainage, low frequencies in the Queen Charlotte sample and at intermediate frequencies over much of the northern half of the Province. Haplotype 1 may have expanded from a Queen Charlotte refuge, though it is presently at low frequency there. Expansion of haplotype 1 from the Columbia into the Fraser seems likely (see above). An alternative source for this haplotype in northern rivers (e.g. Peace, Skeena) is crossover from the Fraser. As recolonization from the coast was likely to be earlier than expansion from an interior southern refuge, trout from the south interior may have expanded into this region after coastal trout were present. As mtDNA introgresses more easily than nuclear DNA, mtDNA is more likely to remain in this population than nuclear DNA. Crossovers among interior river systems are thought to have occurred during recolonization (McPhail and Lindsey, 1986), and expansion throughout the province from an interior refuge has been previously hypothesized for bull trout (Taylor *et al.*, 1999).

That B.C. coastal rainbow trout do not appear to have come from Beringia contradicts Okazaki (1984) and stands in stark contrast to other salmonid species. Almost all other Pacific salmonids not only survived in a northern refuge, but also can attribute most of their diversity to northern populations. In sockeye and pink salmon, the Skeena River in B.C. is part of the "northern" group (Varnavskaya and Beacham, 1992; Bickam *et al.*, 1995; Taylor *et al.*, 1996). Chum salmon exhibit a contact zone in Alaska between northern and southern populations (Seeb and Crane, 1999). Studies have shown higher genetic diversity in northern populations than southern ones (Cronin *et al.*, 1993), possibly due to a more ancient history in the north or to a larger glacial population in Beringia than the Columbia. The major split in rainbow and steelhead, however, appears not to be between northern and southern populations, but rather between coastal and inland ones. Minisatellite differences (Taylor, 1995) and differences in mtDNA haplotypes suggest that genetic material from the Beringian refuge remain confined to

Alaska and Kamchatka area in rainbow trout.

The main contrast between our findings and previous hypotheses on phylogenetic structure in rainbow trout is the existence of the north coast phylogenetic group (Utter *et al.* 1980, Carl *et al.* 1994). The coastal-inland split demonstrated by allozyme data in the 1970s is still the strongest and sharpest divergence in rainbow trout. Coastal and inland populations, partitioned in various ways, account for 20% of the genetic variation in B.C. (see Table 5). The corroboration of this divergence from both allozymes and minisatellites further supports the significance of this phylogeographic division (Utter *et al.*, 1980; Parkinson, 1984; Taylor, 1995). Morphological studies have also demonstrated differences between the coast and inland (Behnke, 1992). In the south, this boundary is very sharp and corresponds to the Cascade/Coastal mountain crest on both the Fraser and Columbia rivers. Climatic and ecological differences exist between the coastal and interior regions of the province (McPhail and Lindsey, 1986), and, therefore, strong selective forces may be partially responsible for reducing gene flow and maintaining the boundary between phylogenetic groups. Alternatively, geographical barriers such as the Fraser Canyon, although not absolute, may represent at least a partial to gene flow between these groups.

Conservation Recommendations

Historically, conservation programs have focussed on the species as the unit of conservation but, more recently, an appreciation is being gained for the importance of genetic conservation at the population level (Avise 1994, Meffe and Carroll 1994). This is particularly true of salmonids, which have a wide geographic distribution and show considerable population subdivision that is evident in both phenotypic and genotypic variation (Ricker 1972, Taylor 1991). The ultimate focus for the conservation of this diversity is the genetic variation.

Identifying and prioritizing genetic variation within species is increasingly being done with phylogenetic tools (Bernatchez 1995, Taylor and Haas 1996). The deepest genetic divisions within a species are often the result of historical isolation related to range shifts and geological events (Bernatchez and Wilson 1998). These biogeographical groups, therefore, represent significant pool of genetic resources that is available for future adaptive radiation (Allendorf and Leary 1988, Taylor and Haas 1996). The importance of phylogenetic variation lies in its unambiguous genetic nature, which represents the oldest levels of genetic divergence within a species. Phylogenetic groups are presumably distinct throughout the genome and therefore need to be a primary consideration in the conservation of intraspecific genetic variation.

Our recommendation of a phylogenetic classification system is based on a model of source areas and recolonized areas (Fig. 9). Current data suggest that genetic material from three phylogenetic groups is represented in BC rainbow trout populations. Priority is given to the best representatives of source areas and recolonized areas are allocated to separate groups depending on their hypothesized origin or mixture of origins. This model is considerably more complex than the alternative of homogenous areas representing single phylogenetic groups separated by relatively sharp geographic boundaries. The weight of evidence suggests that the complex model more accurately represents the true phylogenetic associations in this species. We recognize that

further sampling may alter or contradict this model but we believe that it represents the best scientific opinion and forms a firm basis for conservation decisions on phylogenetic organization in rainbow trout.

Our approach to classifying populations is based on a series of principles. Locations close to the source areas are assumed to be the most representative of the full range of genetic variation within a phylogenetic group. Conservation of these source areas is intended to capture the deepest subdivisions within the species. Areas that were colonized from a single phylogenetic group also represent deep subdivisions within the species even though a proportion of the alleles present in each group may have been lost during the recolonisation and range expansion process. Finally, areas colonized from 2 or more sources represent the recombination of phylogenetic lines, which may increase the scope for adaptive radiation.

The operational definition of phylogenetic structuring in rainbow trout is outlined in Figure 10. Source areas, which represent glacial refugia, form the core of the classification process. The Queen Charlottes is a key source area since it is entirely within B.C. Source areas located further south are represented in B.C. only where colonists have entered the province (southern Vancouver Island, southern interior) but these regions are geographically close to the original sources. There is some evidence for a further split in the South Interior Group and therefore this group could be further split along watershed boundaries (Columbia vs. Fraser). Much of the north coast and interior appears to represent a colonized area that originated from the Queen Charlotte source area.

The southern B.C. colonized areas are important components of their respective phylogenetic groups because the integrity of the original source populations farther south has been severely compromised by transfers among drainages, exotic species and habitat destruction. These areas are also the most threatened in B.C. but pristine populations are present. In the north, the Skeena and other drainages colonized from the Queen Charlotte refuge are also important components of the Queen Charlotte phylogenetic line. The Queen Charlotte Islands themselves have a relatively limited range of habitats. A much wider range of habitats are represented in the large area colonized from this refuge and this will likely capture a much larger range of adaptive variation for this phylogenetic group.

Multiple-source colonized areas represent unique blends of genetic materials from multiple sources. These areas are unlikely to be homogeneous or have sharp boundaries and represent a difficult problem in classification. The current scheme ignores heterogeneity within these areas and classifies each overlap area as a single group of populations with a uniform phylogenetic history.

Key uncertainties are almost all associated with multiple source areas. Drainages that penetrate the Coast Mountains in southern BC (Kliniklini, Homathko) are given overlap status because some freshwater species from the interior have colonized these drainages (e.g. Taylor *et al.* 1999). There is currently no information on the genetic profile of rainbow trout from these watersheds to justify this designation. More samples are also needed from the central coast, the upper Fraser, and the Peace in order to define the extent of the overlap zones in these areas.

Figure 10. Operational definition of phylogenetic groups within rainbow trout.

Rainbow trout is also well known for its extensive adaptive variation (Taylor, 1991). Both ecotypic and phylogenetic variation are important elements of diversity in any species worth conserving. That phylogenetic variation most likely predates ecotypic variation (see Table 3) in this species, suggests that it deserves a higher priority in a conservation regime. However, within phylogenetic groups, emphasis will be placed on conserving ecotypic variation as much as possible. Particularly unique ecotypes and those characteristics that exhibit a genetic component will be prioritized.

Furthermore, similarity of mtDNA haplotypes among populations does not indicate that the populations have not diverged. Distinct populations may not have had time to differentiate at molecular markers, though their adaptive characteristics may be quite different and contribute significantly to intraspecific genetic diversity (Utter *et al.*, 1993). Thus, phylogenetic and adaptive diversity should be considered complementary forms of diversity and we strongly recommend that adaptive variation be conserved within each phylogenetic group.

In summary, because the future adaptive potential of the species depends on genetic variation, conservation plans should focus on prioritizing levels of genetic variation in the species. The extent and distribution of genetic variation in the province reinforces the importance of Pleistocene glaciations in shaping intraspecific diversity.

Mitochondrial DNA supports a phylogenetic division in rainbow trout into two major clades in British Columbia, a result that is supported by other molecular differences (Utter *et al.*, 1980; Taylor, 1995). The two clades have distinctive geographic distributions in coastal and inland areas although they do overlap in some areas. This phylogenetic variation represents the deepest evolutionary division within rainbow trout and, therefore, conservation plans should prioritize the maintenance of this distinction. Although postglacial dispersal and gene flow may have blurred the geographic separation between the clades to some degree, the corroboration among several phylogenetic systems attests to its historical reality. Prioritizing locations closest to former refugia in a conservation plan is recommended not only because they are likely to contain the most distinct genetic resources in the province, but also because they may well represent the refugia of the future as well. Finally, conservation of adaptive variation is strongly encouraged within these phylogenetic groups, not only because adaptive groups represent genetic diversity, but because during periods of rapid environmental change, survival of the species may depend on existing life history and behavioural variation.

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Appendix 1. Presence-absence matrix of restriction sites found from different restriction enzymes in samples of *O. mykiss*. Restriction sites were inferred from the restriction fragments sizes.

AluI	A	1111111111110111
	B	1111111111111111
	C	1111111111110011
	D	1111111111101011
	E	1111111111011011
AvaII	A	11100
	B	11110
	C	11101
BfaI	A	11111111111111
	B	1111111111101
	C	1111111111011
	D	11111111111110
BstUI	A	1110
	B	1111
DdeI	A	11111111111111111
	B	11111111111111110
	C	11111111111111001
DpnII	A	111111111011
	B	111111110010
	C	111111110100
	D	111111110000
HaeIII	A	1111111111100
	B	1111111111000
	C	1111111110100
	D	1111111110000
	E	1111111111110
	F	1111111111101
	G	1111111101100
HhaI	A	1111111
	B	1111110
HincII	A	111000
	B	111100
	C	111010
	D	111011
HinfI	A	11111111101
	B	11111111111
MspI	A	11111100
	B	11111110
	C	11111101
NlaIII	A	1111111111
	B	1111111110
	C	1111111101
RsaI	A	1111111000
	B	1111111100
	C	1111111110
	D	1111111101
TaqI	A	111111111
	B	111111110

Appendix 2. The 32 composite haplotypes found in *O. mykiss* samples using 14 restriction enzymes. The symbols refer to the digest patterns (Table 4) from the following enzymes:
*Alu*I, *Ava*II, *Bfa*I, *Bst*UI, *Dde*I, *Dpn*II, *Hae*III, *Hha*I, *Hinc*II, *Hin*fI, *Msp*I, *Nla*III, *Rsa*I, *Taq*I

1	A	A	A	A	A	A	A	A	A	A	A	A	A
2	A	A	A	B	A	A	A	A	A	A	A	A	A
3	A	A	A	A	A	A	A	A	A	A	C	A	A	A
4	C	A	A	A	A	A	A	A	A	A	A	A	A
5	B	A	A	A	A	A	A	A	A	A	A	A	A
6	A	A	A	A	A	A	A	A	A	A	C	A	A	A
7	A	A	A	A	A	A	A	D	A	A	A	A	A
8	A	A	A	A	A	A	A	A	A	A	B	A	A	A
9	A	A	A	A	B	A	A	A	A	A	A	A	A
10	A	A	A	A	A	A	F	A	A	A	A	A	A
11	A	A	A	A	A	A	A	A	A	A	A	A	D	A
12	D	A	A	A	A	A	A	A	A	A	A	A	A
13	A	A	A	A	A	B	A	A	A	A	A	A	A
14	A	A	A	A	A	A	A	B	A	A	A	A	A
15	A	A	A	A	A	A	A	A	A	A	A	B	A	A
16	A	A	A	A	A	A	A	A	A	A	A	A	B	A
17	A	A	A	A	A	A	B	A	A	A	A	A	A
18	A	A	A	A	A	A	B	A	A	A	A	B	A	A
19	A	A	A	A	A	A	B	A	A	A	A	A	B	A
20	A	A	A	A	C	A	A	A	C	A	A	A	C	A
21	E	A	C	A	B	A	A	A	A	A	A	A	A
22	A	A	B	A	A	A	E	A	A	A	A	B	A	A
23	A	C	A	A	A	A	G	A	A	B	A	B	A	B
24	A	B	A	A	A	C	C	A	A	B	A	A	A	B
25	A	B	A	A	A	C	C	A	A	B	A	B	A	B
26	A	B	A	A	A	C	C	A	B	B	A	A	A	B
27	A	B	A	A	A	C	A	A	A	B	A	A	A	B
28	A	B	A	A	A	C	D	A	A	B	A	A	A	B
29	C	B	A	A	A	C	C	A	A	B	A	A	A	B
30	E	B	D	A	A	C	C	A	A	B	A	A	A	B
31	B	B	D	A	A	C	C	A	A	B	A	A	A	B
32	A	B	A	A	A	D	C	A	A	B	A	A	A	B

Appendix 3. ND1 sequence samples chosen. The RFLP haplotype and location the sample was chosen from are listed in the first two columns. The corresponding ND1 sequence haplotype is listed in the third column (see Appendix 4 for particular mutations).

RFLP Haplotype	*Sample Location*	*Sequence Haplotype*
1	Kavachina, Kamchatka	RB1
	Wampus Ck, Athabasca R.	
2	Klootchlimmis Ck., Vancouver Island	RB1
3	Quinsam R., Vancouver Island	RB1
5	Babine R., Skeena R.	RB1
6	Alsea R., Oregon	RB1
9	Selway R., Snake R.	RB1
12	Snotolvayam, Kamchatka	RB1
15	Chehalis R., Lower Mainland	RB1
16	Hill Ck., upper Columbia R.	RB3
17	Halfway R., upper Columbia R.	RB1
18	Halfway R., upper Columbia R.	RB1
19	Tumtum Ck., upper Columbia R.	RB3
20	Robertson Ck., Vancouver Island	RB1
21	Selway R., Idaho	RB1
22	Gerrard R., upper Columbia	RB1
23	Dean R., coastal B.C.	RB2
24	Kutcho Ck., Liard River	RB5
26	Honna R., Queen Charlotte Islands	RB4
27	BVM, Queen Charlotte Islands	RB4
28	Kitimat R., coastal B.C.	RB4
29	Honna R., Queen Charlotte Islands	RB4
30	Cheslatta L., upper Fraser	RB6
31	Vedder R., Lower Mainland	RB7
32	Klootchlimmis Ck., Vancouver Island	RB4
	coastal cutthroat, Vancouver Island	CUT1

Appendix 4. Seven ND1 haplotypes found among rainbow trout samples (RB1-7) and one cutthroat haplotype. Mutations are indicated in the table with the entire 468 bp sequence of RB1 listed below. The sequenced is numbered from 1-468, starting at the 5' end with NDB as the primer. RB= rainbow trout, CUT= cutthroat trout.

	21	25	35	60	123	134	135	138	159	201	204	219	231	252	272	276
CUT1	A	T	C	C	A	A	C	G	C	C	A	G	G	C	A	A
RB1	G	C	G	T	G	G	T	G	T	T	A	A	C	T	G	G
RB2	G	C	G	T	G	G	T	A	T	T	A	A	C	T	G	G
RB3	G	C	G	T	G	G	T	G	T	T	G	A	C	T	G	G
RB4	G	C	G	T	G	G	T	G	T	T	A	A	C	C	G	G
RB5	G	C	G	T	G	G	T	G	T	T	A	A	C	T	G	G
RB6	G	C	G	T	G	G	T	G	C	T	A	A	C	C	G	G
RB7	G	C	G	T	G	G	T	G	T	T	A	A	C	T	G	G

	282	296	309	342	357	366	381	384	390	399	411	414	423	435	441	465
CUT1	T	T	G	C	T	C	G	C	T	G	T	T	C	C	T	C
RB1	C	G	A	T	C	T	A	T	T	T	C	T	T	T	G	G
RB2	C	G	A	T	C	T	A	T	T	T	C	T	T	T	G	G
RB3	C	G	A	T	C	T	A	T	T	T	C	T	T	T	G	G
RB4	C	G	A	T	C	T	A	T	T	T	C	C	T	T	G	G
RB5	C	G	A	T	C	T	A	T	C	T	C	C	T	T	G	G
RB6	C	G	A	T	C	T	A	T	T	T	C	C	T	T	G	G
RB7	C	G	A	T	C	T	A	T	T	T	C	C	T	T	G	G

TCTCTTGAGG ATGGGTTCGA GCCCCTTCTC TCTAGAATTG AGGGGACTTG AACCCCTATT
AGCCACGCTA TCAAGGTGGT CCTTAAGCAT TCAGGCACAA TTCCGGGCTA AAGCTGAGGG
GGGAGGCCTG CTAGTGCGAT GGGAAGTGCT AAATGTCATA GTACAAGGGC CAGAGTCAGG
GGTAGGAAGC TTTTCCAAAC TAAATGTATG AGTTGATCAT ACCGAAATCG CGGGTAGGAA
GCTCGTACTC ATAAAAATAC AACGGAGAGG AGGGCGGCTT TCGTTATTAG GTTTAGGGCA
GTTAGTTCAG GGAAAGCAGG GATGTGGGAT GCGCCTAAAA ATAGGACGGC TGAGAGCGTA
TTTATTAGAA GGATATTAGC ATATTCGGCT AGGAAAAATA GGGCAAAGGG CCCTCCAGCA
TATTCTACAT TGAATCCGGA GACTAATTCT GACTCTCCTT CTGTGAGG